Journal of
Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

# Impact of *Aspergillus* section *Flavi* community structure on the development of lethal levels of aflatoxins in Kenyan maize (*Zea mays*)

C. Probst[1], F. Schulthess[2] and P.J. Cotty[1,3]

1 Department of Plant Sciences, The University of Arizona, Tucson, Arizona, USA
2 International Centre of Insect Physiology and Ecology, Plant Health Division, Nairobi, Kenya
3 USDA-ARS, Department of Plant Sciences, The University of Arizona, Tucson, AZ, USA

**Keywords**
aflatoxicosis, *Aspergillus flavus*, corn, fungal community structure, Kenya.

**Correspondence**
Peter J. Cotty, USDA-ARS, Department of Plant Sciences, University of Arizona, Tucson, AZ, USA. E-mail: pjcotty@email.arizona.edu

2009/0737: received 24 April 2009, revised 29 May 2009 and accepted 8 June 2009

doi:10.1111/j.1365-2672.2009.04458.x

## Abstract

**Aims:** To evaluate the potential role of fungal community structure in predisposing Kenyan maize to severe aflatoxin contamination by contrasting aflatoxin-producing fungi resident in the region with repeated outbreaks of lethal aflatoxicosis to those in regions without a history of aflatoxicosis.
**Methods and Results:** Fungi belonging to *Aspergillus* section *Flavi* were isolated from maize samples from three Kenyan provinces between 2004 and 2006. Frequencies of identified strains and aflatoxin-producing abilities were assessed, and the data were analysed by statistical means. Most aflatoxin-producing fungi belonged to *Aspergillus flavus*. The two major morphotypes of *A. flavus* varied greatly between provinces, with the S strain dominant in both soil and maize within aflatoxicosis outbreak regions and the L strain dominant in nonoutbreak regions.
**Conclusions:** *Aspergillus* community structure is an important factor in the development of aflatoxins in maize in Kenya and, as such, is a major contributor to the development of aflatoxicosis in the Eastern Province.
**Significance and Impact of the Study:** Since 1982, deaths caused by aflatoxin-contaminated maize have repeatedly occurred in the Eastern Province of Kenya. The current study characterized an unusual fungal community structure associated with the lethal contamination events. The results will be helpful in developing aflatoxin management practices to prevent future outbreaks in Kenya.

## Introduction

Today, 500 years after its introduction, maize (*Zea mays*) is the most widely grown staple food in Africa (McCann 2005). Maize consumption is a primary avenue through which humans in Africa become exposed to aflatoxins (Egal *et al.* 2005; Shephard 2008). Aflatoxins are metabolites produced by several *Aspergillus* species. These metabolites are highly toxic to humans and domestic animals. To minimize potential human exposure, the aflatoxin content of food and feed is strictly regulated in most of the world (Van Egmond and Jonker 2004; Shephard 2008). However, these standards have little relevance to poor, small-scale farmers in Africa, who often rely on maize for daily nutrition and income.

Aflatoxin contamination of maize may be caused by several species in *Aspergillus* section *Flavi*. These fungi vary widely in both ability to infect and decay crops and aflatoxin-producing capacity (Cotty 1989). Thus, the potential of these fungi to contaminate crops with aflatoxin also varies. Aflatoxin-producing members of sect. *Flavi* also differ in morphology, physiology and ecology (Cotty 1989; Cotty *et al.* 1994; Bock *et al.* 2004). In general, the process of crop contamination with aflatoxins begins in the field during crop development and may continue after crop maturation until the grain is ultimately consumed (Cotty *et al.* 1994). Contamination is strongly influenced by abiotic factors such as temperature and humidity as well as biotic factors including insects and the average aflatoxin-producing potential of the fungal community associated with crops (Cotty 1997; Cotty *et al.* 2008). *Aspergillus parasiticus* and *Aspergillus flavus* are the species most commonly implicated as causal agents of aflatoxin contamination (Klich 2007; Cotty *et al.* 2008). *Aspergillus flavus* is delineated into two morphotypes called the S and L strains (Cotty 1989). The S strain produces many small sclerotia (<400 $\mu$m in diameter), relatively few conidia and consistently high levels of aflatoxin. The L strain produces fewer, larger sclerotia (>400 $\mu$m in diameter), more conidia and, on average, less aflatoxin than the S strain. A significant percent of L strain isolates produce no aflatoxin. Several of these atoxigenic isolates are the principal active agents in biocontrol products that are used to manage aflatoxin contamination (Cotty and Bhatnagar 1994; Dorner 2004a).

The most common aflatoxin, aflatoxin $B_1$, is a genotoxin known to be carcinogenic and teratogenic for both humans and animals (Wang and Tang 2004; Mckean *et al.* 2006). This aflatoxin was first listed as a human carcinogen in the First Annual Report on Carcinogens in 1980 by the National Toxicology Program of the Department of Health and Human Services (NTP 1980). To date, aflatoxin $B_1$ is the only mycotoxin classified as a Group 1a human carcinogen by the International Agency for Research on Cancer (IARC 1982, 2002). Intake of low, daily doses of aflatoxins over long periods may result in chronic aflatoxicosis expressed as impaired food conversion, stunting in children (Gong *et al.* 2004), immune suppression, cancer and reduced life expectancy (Cardwell and Henry 2004; Gong *et al.* 2004; Williams *et al.* 2004; Farombi 2006). Ingestion of high concentrations of aflatoxin results in rapid development of acute aflatoxicosis characterized by severe liver damage leading to jaundice, hepatitis and, when most severe, death (Williams *et al.* 2004). Outbreaks of acute aflatoxicosis have never been reported for developed countries but have occurred in several developing countries (Krishnamachari *et al.* 1975; Tandon *et al.* 1977; Ngindu *et al.* 1982; Lye *et al.* 1995; Probst *et al.* 2007). However, only in India and Kenya have epidemics of acute aflatoxicosis been repeatedly reported.

In Kenya, maize is the staple food that dominates food security considerations. It has a per capita consumption of 98 kg per annum and accounts for about 40% of the daily calorie intake (http://www.fao.org). As a direct consequence, Kenyans are exposed to regular doses of aflatoxins through maize ingestion. The first reported outbreak of acute aflatoxicosis in Kenya occurred in 1982 in the Eastern Province (Ngindu *et al.* 1982). More outbreaks were officially reported in 2001, 2004–2006 and 2008 (Shephard 2003; Anonymous, 2004). The outbreaks occurred exclusively in only 4 of the 71 Kenyan districts. The affected districts are adjacent to each other and located in Kenya's Eastern and Central Provinces. The districts Kitui (Eastern), Machakos (Eastern), Makueni (Eastern) and Thika (Central) were affected, with Kitui and Machakos reporting the highest death rates in all years. Kenya is the only African nation with recurrent outbreaks of acute aflatoxicosis. The 2004 outbreak was one of the most severe episodes of human aflatoxin poisoning in history and was caused by ingestion of homegrown maize (Lewis *et al.* 2005; Muture and Ogana 2005). Analysis of maize samples collected during the 2004 outbreak by the National Public Health Laboratory Services in Nairobi and the Center for Disease Control and Prevention (CDC) suggests that the fungal community structure was an underlying contributor to the 2004 aflatoxicosis outbreak (Probst 2005; Probst *et al.* 2007). The primary causal agent was determined to be the S strain of *A. flavus*. These conclusions were supported by the high frequency of S strain isolates in highly contaminated maize, by the consistently high aflatoxin production by these S strain isolates *in vitro* and *in vivo*, and by the strong positive correlation between percentage of the S strain in the infecting *A. flavus* community and the maize aflatoxin content (Probst *et al.* 2007). This study was the first to link a particular fungal taxon to an aflatoxicosis epidemic. Identification of the precise causal agent is an important initial step in the development of management practices (Cotty *et al.* 2008). However, information on how fungal communities vary between districts with and without histories of acute aflatoxicosis is unknown, and corroborating evidence for dominance of the S strain on maize from the affected districts in years other than 2004 is lacking.

This study sought to compare communities of aflatoxin-producing fungi on maize in the affected Kenyan districts during the aflatoxicosis outbreak years of 2005 and 2006 with those previously described for 2004 and with fungal communities on maize in adjacent districts with no histories of aflatoxicosis epidemics. In the process, a body of evidence was developed that convincingly implicates fungal community composition as an important factor predisposing the affected districts to increased incidences of acute aflatoxicosis.

## Materials and methods

### Sampling

In 2005, ground maize and soil samples were collected in Kitui district of Eastern Province, Kenya at locations where lethal aflatoxicosis had been reported (Fig. 1). The Eastern Province is characterized by its semi arid

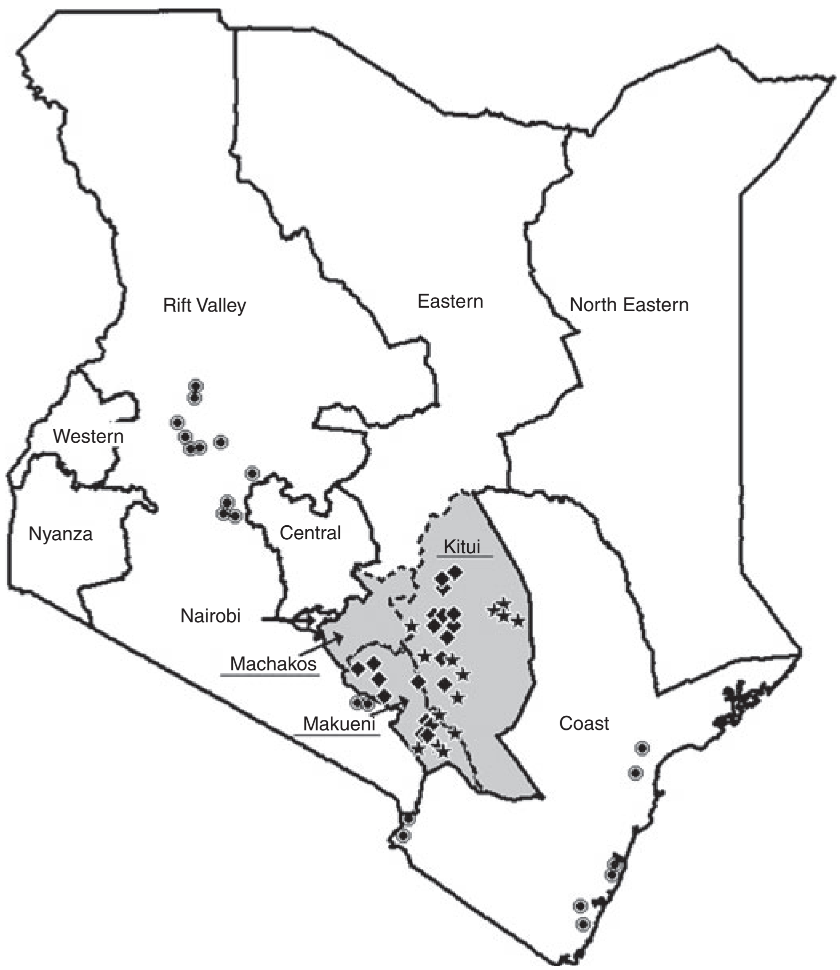


**Figure 1** Map of Kenya indicating sample sites. Districts of the Eastern Province that had reported aflatoxicosis outbreaks are highlighted in grey. Names of districts are underlined. Names of provinces are not underlined. Each symbol may stand for more than one sample. (★) Sample sites in 2005, aflatoxicosis districts; (◆) sample sites in 2006, aflatoxicosis districts and (◉) sample sites in 2006, non-aflatoxicosis provinces.

midlands and bimodal rain patterns. The elevation of this Province is between 400 and 1800 m. Maize samples were taken from household storage, and soil was collected in the fields in which the sampled maize was produced. Each soil sample was a composite of 8–16 subsamples taken from the top 2 cm of soil from locations at least 4 m apart. Additional maize samples were collected from farmers and local markets in Kitui. In 2006, additional ground maize samples were collected in the Eastern Province (Makueni and Kitui districts) (Fig. 1) and in two provinces, the Rift Valley Province and the Coast Province, adjacent to the Eastern Province but with no history of lethal aflatoxicosis (Fig. 1). Six of the Coast Province samples were taken along the coast at elevations between 12 and 145 m. Two samples were taken inland along the maize supply route from Tanzania at about 1000 m. Those areas differ in climate and maize production. The southern coast is warm and semi-humid with bimodal rain patterns and some maize production. In contrast, the north coast is warmer and dryer with very little maize production but high import rates. All maize samples from the Rift Valley originated from high elevation areas (1026–2412 m) in the central and eastern parts of the province (Fig. 1). The central region of the Rift Valley is characterized by its cool and humid climate with only one long rain season and intensive maize production. Most of the maize for local markets and export is grown in this area. The humid eastern parts have a bimodal rain pattern and sufficient maize production for local consumption. Soil and maize samples were imported to the USDA, ARS, Laboratory for Aflatoxin Reduction in Crops, at the University of Arizona, Tucson under permits issued by the USDA Animal and Plant Health Inspection Service.

### Culture medium

Modified rose Bengal agar (M-RB), a defined, semi-selective medium for *Aspergillus* sect. *Flavi* (Cotty 1994), was used for isolations. For culture maintenance, 5/2 agar

(5% V8-juice, 2% agar, adjusted to pH 5·2 prior autoclaving) was used.

### Fungal isolation and quantification

Prior to analysis, maize and soil samples were homogenized. Soil samples were hammered to break up soil clods prior to homogenization. Maize samples were finely ground in a laboratory mill. Both ground maize and powdered soil were vigorously shaken to ensure proper mixing. Samples were also weighed, analysed for moisture content (HB43 Halogen Moisture Analyzer; Mettler Toledo, Columbus, OH), dried to 5–8% moisture to prevent fungal growth and stored for up to 4 weeks at 4°C until further analysis. Maize samples were between 110 and 433 g (mean = 291 g).

Fungal isolates were recovered by dilution plate technique on M-RB (Cotty 1994). Sample material (about 1 g) was mixed by inverting in a 15-ml test tube containing 5 ml sterile-distilled water for *c.* 20 min, and aliquots (100 $\mu$l per plate) of the resulting suspension were spread on M-RB plates ($n = 3$). After incubation (3 days, 31°C, dark), *Aspergillus* sect. *Flavi* colonies were enumerated [Colony Forming Units (CFU) per g]. Up to 10 discrete colonies were aseptically transferred to 5/2-agar and incubated (5–7 days, 31°C). *Aspergillus* species (Kurtzman *et al.* 1987; Klich and Pitt 1988) and strains (Cotty 1989) were identified by both macroscopic and microscopic characters. Isolations were performed two to four times to verify results. A total of 15 *Aspergillus* sect. *Flavi* isolates were stored long term as 3-mm plugs of sporulating culture in sterile-distilled water at 4°C. Because *Aspergillus tamarii* has been repeatedly reported to be atoxigenic, isolates of *A. tamarii* were identified, enumerated and discarded after initial verification of the atoxigenicity of *A. tamarii* isolates from Kenya.

### Quantification of aflatoxins in ground maize

A USDA/GIPSA certified Enzyme-Linked ImmunoSorbent Assay (ELISA; MycoChek; Strategic Diagnostics, Inc, Newark, DE, USA) was used to detect and quantify aflatoxins in the maize samples. Ground maize samples were mixed thoroughly, and a 50 g sub-sample was blended with 250 ml 70% aqueous methanol, and the aflatoxin content determined according to the manufacturer's instructions.

### Aflatoxin production in maize kernels

The aflatoxin-producing abilities of 126 random L strain isolates that originated from three Kenyan provinces in 2006 were compared. An aflatoxin assay in maize kernels was conducted to assess toxin production in the host. The experiment was conducted twice, with 84 isolates in the first experiment and 42 isolates in the second experiment, and aflatoxin values were calculated as an average from three repetitions. Undamaged maize kernels (10 g 250 ml$^{-1}$ Erlenmeyer flask) were autoclaved (60 min), cooled to room temperature and adjusted to 25% moisture. Flasks were seeded with freshly prepared spore suspensions (2 ml containing $1{\cdot}9$–$2{\cdot}0 \times 10^6$ spores) from 5-day-old cultures and incubated for 7 days at 31°C in the dark. Maize cultures were blended in 80% methanol (50 ml) until evenly homogenized and maize–methanol slurry was filtered through Whatman No. 4 paper. Culture filtrate was spotted directly onto thin-layer chromatography (TLC) plates (Silica gel 60; EMD, Darmstadt, Germany) adjacent to aflatoxin standards (Aflatoxin Mix kit-M; Supelco Bellefonte, PA) containing a mixture of aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$. Plates were developed in ethyl ether–methanol–water, 96 : 3 : 1, air-dried, and aflatoxins were visualized under 365-nm UV light. Aflatoxins were quantified directly on TLC plates with a scanning densitometer (TLC Scanner 3; Camag Scientific Inc, Wilmington, NC, USA). Filtrates initially negative for aflatoxins were partitioned twice with methylene chloride and concentrated prior to quantification (limit of detection 1 ng g$^{-1}$ mycelium) as previously described (Cotty 1997). Each isolate was subjected to three replications, and each experiment was performed twice.

Production of spores by *A. flavus* isolates on maize kernels was determined with a turbidity meter (Model 965-10; Orbeco-Hillige, Farmingdale, NY, USA). After inoculation and growth as described earlier, kernels were washed with 50 ml methanol, 1 ml of the resulting spore suspension was diluted in 19 ml EtOH : $H_2O$, the turbidity measured, and spore concentration calculated with the Nephelometric Turbidity Unit (NTU) *vs* CFU curve $Y = 49\,937X$ ($X$ = NTU, $Y$ = spores per ml).

### Data analysis

Mean comparisons were subjected to either Student's *t*-test or, for multiple comparisons, Analysis of Variance and Tukey's HSD test as implemented in either Stata 9.2 (StataCorp, College Station, TX, USA) or sas 8.0 (SAS Institute, Cary, NC, USA).

## Results

### Isolation and quantification of *Aspergillus* sect. *Flavi* from ground maize

In the current study, 2256 isolates of *Aspergillus* sect. *Flavi* were examined from a total of 165 ground maize samples

**Table 1** Incidences of *Aspergillus* section *Flavi* species and strains on maize grown in three provinces of Kenya

| Sampling year | Kenyan province | Kenyan district | Aflatoxicosis outbreaks | Number of samples | Number of isolates | Total aflatoxin ($\mu$g kg $^{-1}$) | *Aspergillus flavus* S strain (%) | *Aspergillus flavus* L strain (%) | *Aspergillus parasiticus* (%) | *Aspergillus tamarii* (%) | Other (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2005 | Eastern | Kitui | Yes | 39 | 585 | 426·3[a] | 83[a] | 15[a] | 2[a] | 0[a] | 0[a] |
| 2006 | Eastern | Kitui | Yes | 45 | 540 | 219·6[a] | 75[a] | 25[a] | 0[a] | 0[a] | 0[a] |
| 2006 | Eastern | Makueni | Yes | 60 | 791 | 375·9[a] | 70[a] | 25[a] | 4[a] | 0[a] | 1[a] |
| 2006 | Coast | Taita taveta | No | 2 | 37 | 0·1[b] | 0[b] | 81[b] | 0[a] | 19[a] | 0[a] |
| 2006 | Coast | Kwale | No | 2 | 40 | 120·4[b] | 0[b] | 81[b] | 0[a] | 19[a] | 0[a] |
| 2006 | Coast | Tana river | No | 2 | 32 | 10·9[b] | 0[b] | 90[b] | 0[a] | 10[a] | 0[a] |
| 2006 | Coast | Kilifi | No | 2 | 30 | 1·8[b] | 0[b] | 100[b] | 0[a] | 0[a] | 0[a] |
| 2006 | Rift Valley | Marakwet | No | 2 | 32 | 0[b] | 13[b] | 84[b] | 0[a] | 0[a] | 3[a] |
| 2006 | Rift Valley | Baringo | No | 3 | 47 | 0[b] | 2[b] | 88[b] | 0[a] | 2[a] | 8[a] |
| 2006 | Rift Valley | Keiyo (ii) | No | 2 | 30 | 13·4[b] | 0[b] | 90[b] | 0[a] | 0[a] | 10[a] |
| 2006 | Rift Valley | Kajiado | No | 2 | 31 | 6·6[b] | 3[b] | 90[b] | 0[a] | 7[a] | 0[a] |
| 2006 | Rift Valley | Nakuru | No | 2 | 31 | 5·6[b] | 0[b] | 97[b] | 0[a] | 3[a] | 0[a] |
| 2006 | Rift Valley | Laikipia | No | 2 | 32 | 3·1[b] | 0[b] | 94[b] | 0[a] | 3[a] | 3[a] |

Total aflatoxin (ppb), sum of aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ in the maize sample; S strain (%), percentage of *Aspergillus* sect. *Flavi* isolates belonging to the S strain of *A. flavus*; L strain (%), percentage of *Aspergillus* sect. *Flavi* isolates belonging to the L strain of *A. flavus*; *A. parasiticus* (%), percentage of *Aspergillus* sect. *Flavi* isolates belonging to *A. parasiticus*; *A. tamarii* (%), percentage of *Aspergillus* sect. *Flavi* isolates belonging to *A. tamarii*; other (%), percentage of isolates for which species could not be assigned.
Means followed by the same letter in each column are not significantly different ($P < 0{\cdot}05$) by Tukey's Studentized Range test.

obtained in 2005 and 2006 (Table 1). Results for 2004 were previously reported (Probst *et al.* 2007). In total, *A. flavus* made up 98% of *Aspergillus* sect. *Flavi* isolates from maize samples that originated in the affected Kenyan districts. On the basis of colony characteristics and sclerotial morphology, 76% of the *A. flavus* isolates from the affected areas belonged to the S strain morphotype and 22% to the L strain morphotype. Incidences of the morphotypes did not differ ($P = 0{\cdot}05$) among 2004, 2005 and 2006. *A. parasiticus* was only present in 26 samples and made up 2% of the isolates. Other members of *Aspergillus* sect. *Flavi* made up <1% of the total isolates (Table 1).

In stark contrast to the Eastern Province, maize samples from the Coast Province ($n = 8$) mainly contained the L strain of *A. flavus* (88% of *Aspergillus* sect. *Flavi*), and no S strain isolates were recovered. The remaining 12% of *Aspergillus* sect. *Flavi* isolates were the atoxigenic species *A. tamarii* (Table 1). Maize from the Rift Valley was also predominantly infected by *A. flavus* (mean = 94%) with the L strain dominant (mean = 91%) and with the S strain composing up 13% of the *Aspergillus* sect. *Flavi* fungi (mean = 3%). Other *Aspergillus* sect. *Flavi* species were minor components of the examined fungal communities (Table 1). *Aspergillus parasiticus* was not detected in either the Coast or Rift Valley Provinces (Table 1). Compositions of *A. flavus* communities associated with maize from the aflatoxicosis outbreak region differed significantly ($P < 0{\cdot}05$) from those associated with both the Rift Valley and Coast Provinces (Table 1).

### *Aspergillus* sect. *Flavi* in paired soil and maize samples from small stakeholder farms in Kitui district, Eastern Province

Fifteen *Aspergillus* sect. *Flavi* isolates were recovered from each maize and soil sample (14 pairs total) from affected households in Kitui district. The only aflatoxin-producing species detected were *A. flavus* and *A. parasiticus*. The latter species was present in only one maize and six soil samples where it composed 0·5–13% of the *Aspergillus* sect. *Flavi*. *Aspergillus flavus* was the most common aflatoxin producer in all 28 samples. In maize, 91·3% of the *Aspergillus* sect. *Flavi* isolates belonged to the S strain and 8·3% to the L strain; only 0·5% belonged to *A. parasiticus*. Incidence of the S strain was significantly ($P < 0{\cdot}001$) less (61% *vs* 91%) in soil than in maize while incidences of both the L strain and *A. parasiticus* were greater (fig. 2). Additionally, the nonaflatoxin-producing species *A. tamarii* was present in 9% of the soil samples, but was not detected in any maize sample. There were significantly more ($P < 0{\cdot}005$, Student *t*-test) *Aspergillus* sect. *Flavi* propagules in maize (mean = 487 CFU $g^{-1}$) than in the soil (mean = 9·4 CFU $g^{-1}$).


Journal compilation © 2009 The Society for Applied Microbiology, Journal of Applied Microbiology **108** (2010) 600–610
No claim to US Government works

**Table 2** Comparison of aflatoxin and spore production by *Aspergillus flavus* L strain isolates from three adjacent provinces in Kenya

| Kenyan province | Experiment no. | Number of tested L strain isolates | Avg. aflatoxin $B_1$ ($\mu$g kg$^{-1}$) | Atoxigenic (%) | Avg. spores per ml |
|---|---|---|---|---|---|
| Eastern | 1 | 28 | 13 200[a] | 54 | $1{\cdot}2 \times 10^{7a}$ |
| Rift Valley | 1 | 28 | 12 000[a] | 61 | $1{\cdot}4 \times 10^{7a\ b}$ |
| Coast | 1 | 28 | 1200[b] | 82 | $1{\cdot}5 \times 10^{7b}$ |
| Eastern | 2 | 14 | 27 800[a] | 57 | $1{\cdot}2 \times 10^{7a}$ |
| Rift Valley | 2 | 14 | 12 200[a] | 79 | $1{\cdot}3 \times 10^{7a}$ |
| Coast | 2 | 14 | 4800[b] | 93 | $1{\cdot}6 \times 10^{7b}$ |

Means followed by the same letter in each column are not significantly different ($P < 0{\cdot}05$) by Tukey's Studentized Range Test. Different isolates were used in the two experiments.
Avg., average value of three repetitions.

### Aflatoxin content in maize

Aflatoxin content in maize, as determined by ELISA, differed significantly ($P = 0{\cdot}01$, Student's *t*-test) among provinces with the greatest concentrations of aflatoxins found in maize from the Eastern Province (mean = 340 ppb, range of annual means = 219–426). The aflatoxin content of maize from the Eastern Province did not differ significantly between 2005 and 2006 (Table 1). Only 41% of the Eastern Province samples were below 20 ppb compared to 75% percent of maize from the Coast and 100% of the samples from the Rift Valley (Table 1). Only one sample recovered from Kwale district in the Coast Province was highly contaminated with 240 ppb total aflatoxin.

### Aflatoxin production in maize kernels

Aflatoxin-producing ability of L strain isolates from three Kenyan provinces (total of 42 isolates per district) was assessed on maize kernels (Table 2). The results obtained from both experiments were consistent with each other. L strain isolates from Eastern Province and Rift Valley Province did not differ significantly. Both aflatoxin production and sporulation on maize were similar. On the other hand, L strain isolates from the Coast Province consistently produced the lowest concentration of aflatoxin $B_1$ and had the highest incidence of atoxigenic strains (Table 2). Isolates from the Coast Province also produced significantly ($P < 0{\cdot}05$) more spores on maize than isolates from the Eastern Province, but differences with isolates from the Rift Valley were only detected in the second experiment.

Aflatoxin $B_1$ production by 22 toxigenic *A. flavus* isolates from the Eastern Province (11 L strain and 11 S strain isolates) was compared (Table 3). Isolates were obtained from maize collected in the Eastern Province in 2004. Aflatoxin $B_1$ production by L and S strains differed significantly ($P < 0{\cdot}001$). L strain isolates produced 4 ppb to 15 ppm aflatoxin $B_1$. In contrast, S

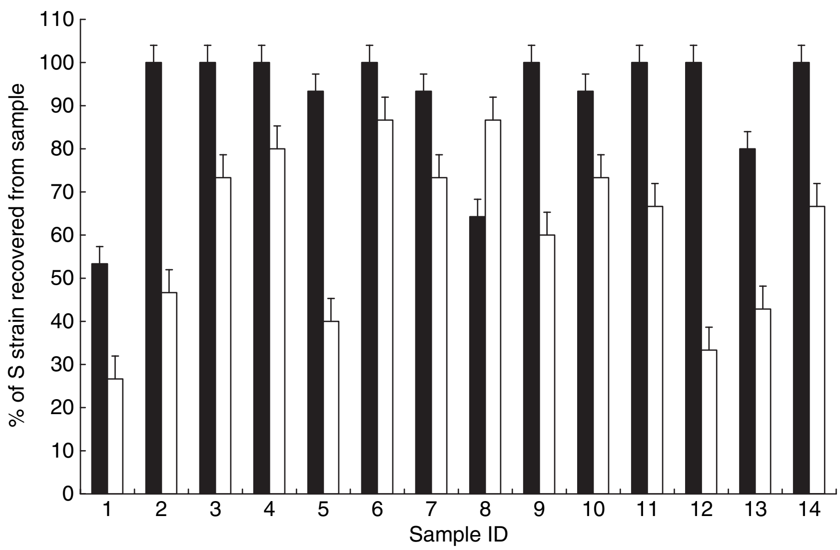


**Figure 2** Per cent of *Aspergillus* sect. *Flavi* composed of the S strain in maize (black bars) and soil samples (white bars) obtained at 14 locations in Kitui district (Eastern Province, Kenya) in 2005. Error bars indicate standard errors of the mean. The means of the maize and soil samples were significantly different at the $P < 0{\cdot}001$ (paired *t*-test).

**Table 3** Aflatoxin $B_1$ production on maize kernels by *Aspergillus flavus* S and L strain isolates from the Eastern Province of Kenya

| | Average aflatoxin $B_1$ ($\mu$g kg$^{-1}$) | |
|---|---|---|
| Isolate no. | *A. flavus* L strain | *A. flavus* S strain |
| 1 | $4^{c}$ | $7520^{h}$ |
| 2 | $6^{c}$ | $14\ 666^{h}$ |
| 3 | $9^{c}$ | $18\ 368^{h}$ |
| 4 | $16^{c}$ | $34\ 096^{h}$ |
| 5 | $6261^{b,c}$ | $59\ 680^{g,h}$ |
| 6 | $6411^{b,c}$ | $101\ 151^{f,g}$ |
| 7 | $6950^{b}$ | $115\ 725^{f,g}$ |
| 8 | $7263^{b}$ | $126\ 047^{e,f}$ |
| 9 | $7361^{a,b}$ | $136\ 896^{e,f}$ |
| 10 | $10\ 946^{a}$ | $179\ 243^{d,e}$ |
| 11 | $15\ 108^{a}$ | $233\ 029^{d}$ |
| Mean | 5485 | 93 311 |

Values (averages of three replicates) followed by the same letter are not significantly different ($P = 0{\cdot}05$) from each other (Tukey–Kramer HSD test). L and S strain means differ significantly ($P < 0{\cdot}001$, *t*-test).

strain isolates produced up to 233 ppm aflatoxin $B_1$ (Table 3).

## Discussion

The lethal aflatoxicosis outbreak in the Eastern Province of Kenya in 2004 resulted in widespread interest within the international food safety community. Although epidemiological explanations for the contamination were discussed (Azziz-Baumgartner *et al.* 2005; Lewis *et al.* 2005), efforts to precisely describe the etiologic agent lagged until an association of the S strain *of A. flavus* with the most severely contaminated maize was found (Probst *et al.* 2007). The precise aetiology of aflatoxin-contamination events is difficult to describe because aflatoxin-producing fungi exist in communities composed of individuals that vary widely in both virulence to plants and aflatoxin-producing ability (Cotty 1989; Brown *et al.* 1992; Shieh *et al.* 1997; Cotty *et al.* 2008). Thus, both the incidence of a particular fungus in the affected crop and the aflatoxin-producing capacity of the fungus must be taken into consideration. The S strain of *A. flavus* was both very common in maize associated with the 2004 epidemic and capable of producing very high concentrations of aflatoxins. Aflatoxin content of the maize was directly correlated with the proportion of the infecting fungi belonging to the S strain (Probst *et al.* 2007). The current study supports attribution of the S strain as the primary cause of the aflatoxicosis outbreaks in Kenya by describing dominance of the *A. flavus* S strain among fungi infecting maize in regions, where aflatoxicosis outbreaks were reported during 2005 and 2006 (Table 1). Furthermore maize from neighbouring provinces (Rift Valley and Coast) without histories of lethal aflatoxicosis (Fig. 1) was shown to have low (Rift Valley Province) to no (Coast Province) incidences of the *A. flavus* S strain. Aflatoxin production assays confirmed high aflatoxin-producing potentials of S strain isolates from Kenya. All isolates consistently produced much higher quantities of aflatoxin $B_1$ than L strain isolates (averages = 93 *vs* 548 ppm, Table 3). This result is similar to observations from other continents (Saito *et al.* 1986; Novas and Cabral 2002). S strain incidence was previously correlated with crop aflatoxin content (Jaime-Garcia and Cotty 2006a).

To determine whether L strain isolates contribute similarly to aflatoxin-producing potential of fungal communities within and adjacent to outbreak areas, we compared aflatoxin production by L strain isolates from each of the three Kenyan provinces studied. L strain isolates from the Coast Province produced less aflatoxin than those from either the Eastern or Rift Valley Provinces. Reduced aflatoxin-producing potential in the Coast Province was associated with high frequencies (93%) of atoxigenic isolates. No S strain isolates were found in Coast Province, and the only other member of *Aspergillus* sect. *Flavi* found was *A. tamarii*, an atoxigenic species. Although L strain isolates did vary in aflatoxin-producing ability among the districts, average aflatoxin production by L strain isolates from all three provinces was consistently below that observed for S strain isolates.

When environmental conditions favour contamination, crops become associated with and infected by complex communities of aflatoxin-producing and closely related fungi (Horn 2003; Cotty and Jaime-Garcia 2007; Cotty *et al.* 2008). Even when only *A. flavus* is present, individual seeds become infected with multiple strains and/or vegetative compatibility groups that vary in aflatoxin-producing capacity (Novas and Cabral 2002; Pildain *et al.* 2004). Atoxigenic strains typically make up significant percentages (Horn and Dorner 1999; Vaamonde *et al.* 2003; Atehnkeng *et al.* 2007; Donner *et al.* 2009) of infecting *A. flavus* communities and greatly modulate the extent to which crops become contaminated (Cotty *et al.* 2008). Indeed, this is one mechanism through which atoxigenic strain biocontrol agents reduce contamination in treated crops. (Cotty and Bayman 1993; Dorner 2004b). The dominance of the S strain and the paucity of atoxigenic *A. flavus* L strain isolates are the most likely explanation for the very high levels of aflatoxin seen in the affected districts of the Eastern Province.

Factors that lead to dominance of the S strain in this area remain unclear. Cultural practices during cultivation, harvest and storage and/or climatic factors may support this dominance, but roles of specific factors need to be investigated.

In addition to aflatoxin, several other highly toxic compounds are known to be concentrated within sclerotia of *Aspergillus* (Wicklow and Cole 1982). Isolates of the S strain produce greater quantities of sclerotia than other *A. flavus* (Jaime-Garcia and Cotty 2004), and S strain sclerotia may form both on crop surfaces and within developing seeds. Sclerotia, particularly those formed within crop tissues, might not be readily evident during hand sorting. Furthermore, during milling, the tiny S strain sclerotia would be cryptically incorporated into the flour. As such, sclerotial production by S strain isolates might contribute toxicity beyond that expected from aflatoxins alone.

Currently, management is directed at cultural practices (i.e. harvest procedures, irrigation and storage) and development of resistant cultivars (Brown *et al.* 2001; Bruns 2003; Turner *et al.* 2005; Kaaya and Kyamuhangire 2006). The identification of the causal fungi may be an initial step in interrupting the aflatoxin contamination processes in Kenya. The S strain of *A. flavus* is ecologically and physiologically different from other aflatoxin producers (Cotty and Mellon 2006) and responds to crop rotations and seasons differently than the L strain isolates (Bock *et al.* 2004; Jaime-Garcia and Cotty 2006b). Thus, the S strain life cycle should be taken into consideration when designing interventions. Furthermore, it is not clear that cultivars respond similarly to L and S strain isolates. When screening cultivars for reduced susceptibility to contamination, incorporating the actual causal agent into screens would be the wisest course. Alternative methods of management should also be considered including use of atoxigenic strains of *A. flavus* as biocontrol agents.

The aflatoxin-producing potential of fungal communities can be reduced through application of native atoxigenic strains of *A. flavus* (Brown *et al.* 1991; Cotty and Bayman 1993; Dorner 2004b). Two atoxigenic strains are registered for aflatoxin management in the United States (Dorner 2004a; Ehrlich and Cotty 2004), and certain atoxigenic strains are known to be particularly effective against the S strain (Garber and Cotty 1997; Cotty and Antilla 2003). Atoxigenic strain applications shift fungal community composition towards dominance of atoxigenic fungi and, as a direct consequence, reduce the aflatoxin content of infected crops (Cotty and Bayman 1993; Dorner *et al.* 1999; Cotty *et al.* 2008). Implementation of biocontrol techniques for West Africa is currently under development (Bandyopadhyay *et al.* 2005; Atehnkeng *et al.* 2008; Cotty *et al.* 2008).

Aflatoxin contamination of maize in Coast Province was detected in the current study at levels considered to be unsafe for human consumption. Thus, the current results indicate that the environment of Coast Province is sufficient to support contamination of maize to unsafe levels by fungal communities lacking the S strain and with relatively low aflatoxin-producing potentials. Establishment of the S strain in maize-producing areas of the Coast Province could be expected to result in increased incidences and severities of contamination.

L strain isolates from the Coast Province produce more spores on maize kernels than L strain isolates from districts affected by acute aflatoxicosis. High sporulating isolates from the Coast Province may be well suited to competitively exclude the S strain during maize production and, as such, atoxigenic isolates among these may be good candidates for biocontrol agents directed at preventing future episodes of lethal aflatoxicosis in the Eastern Province through competitive exclusion of the S strain.

In West Africa, an unnamed taxon (frequently called strain $S_{BG}$) absent from North America but morphologically similar to the S strain of *A. flavus* is common (Cotty and Cardwell 1999). DNA-based phylogenies indicate that strain $S_{BG}$ is a distinct species that groups outside both *A. flavus* and *A. parasiticus* (Egel *et al.* 1994; Ehrlich *et al.* 2005). Morphological similarities between the S strain and strain $S_{BG}$ make differentiation based on macroscopic or microscopic characteristics intractable. However, the *A. flavus* S strain can be readily separated from strain $S_{BG}$ by aflatoxin production. All strains of *A. flavus* produce only B aflatoxins as a result of a 0·8–1·5-kb deletion in the 28 gene aflatoxin biosynthesis cluster (Ehrlich and Cotty 2004). In contrast, strain $S_{BG}$ produces both B and G aflatoxins. Both strain $S_{BG}$ and *A. flavus* are common within communities of aflatoxin-producing fungi in West Africa. However, all *A. flavus* isolates from West Africa belong to the L strain morphotype (Cotty and Cardwell 1999; Cardwell and Cotty 2002; Atehnkeng *et al.* 2008; Donner *et al.* 2009). Indeed, the *A. flavus* S strain had not been detected in Africa prior to the initial report on maize produced in the Eastern Province of Kenya during 2004 (Probst *et al.* 2007). Our current findings support these observations. Strain $S_{BG}$ was not isolated from any maize or soil samples collected from the Eastern Province in Kenya in 2005 or 2006. *Aspergillus flavus* was the dominant species throughout Kenya with the S strain dominant in the Eastern Province and the L strain dominant in the Coast and Rift Valley Provinces.

In the Eastern Province, the S strain was a more important component of the *A. flavus* community infecting maize than the *A. flavus* community resident in the soil in which the maize was produced (91% of *A. flavus* in maize was S strain, whereas 61% was S strain in soil). This is surprising because the S strain produces relatively few spores compared to the L strain during maize infection. Apparently, in the Eastern Province, there are factors that favour S strain movement to maize from soil and subsequent colonization and infection.

It is not clear from where the *A. flavus* S strain isolates originated. The possibility that they were introduced into Kenya, as was maize, from the Americas should be investigated. It is possible that when maize was distributed over the world, the S strain was also inadvertently introduced in several regions. Similar introductions of pests of maize in Kenya have been reported in the literature. For example, the larger grainborer *Prostephanus truncates* (Horn) (Coleoptera: Bostrichidae) was introduced from Meso-America into East Africa in the early 1980s and has been reported from maize deficit areas in the dry mid-altitudes of Kenya since the early 1990s (Hodges *et al.* 1983, 1996).

Incidences of the *A. flavus* S strain remained high from 2004 to 2006 in the Eastern Province, a period during which outbreaks of acute aflatoxicosis recurred leading to hundreds of deaths. Intervention is urgently needed.

## Acknowledgements

We thank Ross Bagwell for the district map of Kenya; Lauren Lewis, the Kenya Aflatoxicosis Investigation Group and Gerphas Okuku Ogola for supplying samples and data, and members of the Laboratory for Aflatoxin Reduction in Crops for excellent assistance.

## References

Anonymous (2004) Outbreak of aflatoxin poisoning – eastern and central provinces, Kenya, January–July 2004. *MMWR Morb Mortal Wkly Rep* **53**, 790–793.

Atehnkeng, J., Ojiambo, P.S., Donner, M.T.I., Sikora, R.A., Cotty, P.J. and Bandyopadhyay, R. (2007) Distribution and toxigenicity of Aspergillus species isolated from maize kernels from three agro-ecological zones in Nigeria. *Int J Food Microbiol* **122**, 74–84.

Atehnkeng, J., Ojiambo, P.S., Ikotun, T., Sikora, R.A., Cotty, P.J. and Bandyopadhyay, R. (2008) Evaluation of atoxigenic isolates of *Aspergillus flavus* as potential biocontrol agents for aflatoxin in maize. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* **25**, 1264–1271.

Azziz-Baumgartner, E., Lindblade, K., Gieseker, K., Schurz Rogers, H., Kieszak, S., Njapau, H., Schleicher, R., Mccoy, L.F. *et al.* (2005) Case–control study of an acute aflatoxicosis outbreak – Kenya-2004. *Environ Health Perspect* **113**, 1779–1783.

Bandyopadhyay, R., Kiewnick, S., Atehnkeng, J., Donner, M., Cotty, P.J. and Hell, K. (2005) Biological control of aflatoxin contamination in maize in Africa. Abstr. Tropentag 2005. *Conference on International Agricultural Research for Development*. Germany: Stuttgart-Hohenheim.

Bock, C.H., Mackey, B. and Cotty, P.J. (2004) Population dynamics of *Aspergillus flavus* in the air of an intensively cultivated region of south-west Arizona. *Plant Pathol* **53**, 422–433.

Brown, R.L., Cotty, P.J. and Cleveland, T.E. (1991) Reduction in aflatoxin content of maize by atoxigenic strains of *Aspergillus flavus*. *J Food Prot* **54**, 623–626.

Brown, R.L., Cleveland, T.E., Cotty, P.J. and Mellon, J.E. (1992) Spread of *Aspergillus flavus* in cotton bolls, decay of intercarpellary membranes, and production of fungal pectinases. *Phytopathology* **82**, 462–467.

Brown, R.L., Chen, Z.Y., Menkir, A., Cleveland, T.E., Cardwell, K., Kling, J. and White, D.G. (2001) Resistance to aflatoxin accumulation in kernels of maize inbreds selected for ear rot resistance in West and Central Africa. *J Food Prot* **64**, 396–400.

Bruns, H. (2003) Controlling aflatoxin and fumonisin in maize by crop management. *J Toxicol Toxin Rev* **22**, 153–173.

Cardwell, K. and Cotty, P.J. (2002) Distribution of *Aspergillus* section Flavi among field soils from the four agroecological zone of the Republic of Benin, West Africa. *Plant Dis* **86**, 434–439.

Cardwell, K.F. and Henry, S.H. (2004) Risk of exposure to and mitigation of effect of aflatoxin on human health: a West African example. *Toxin Rev* **23**, 217–247.

Cotty, P.J. (1989) Virulence and cultural characteristics of two *Aspergillus flavus* strains pathogenic on cotton. *Phytopathology* **79**, 808–814.

Cotty, P.J. (1994) Comparison of four media for isolation of *Aspergillus flavus* group fungi. *Mycopathologia* **125**, 157–162.

Cotty, P.J. (1997) Aflatoxin-producing potential of communities of *Aspergillus* section Flavi from cotton producing areas in the United States. *Mycol Res* **101**, 698–704.

Cotty, P. and Antilla, L. (2003) *Managing Aflatoxins in Arizona*. New Orleans: United States Department of Agriculture, Agricultural Research Service.

Cotty, P.J. and Bayman, P. (1993) Competitive-exclusion of a toxigenic strain of *Aspergillus-Flavus* by an atoxigenic strain. *Phytopathology* **83**, 1283–1287.

Cotty, P.J. and Bhatnagar, D. (1994) Variability among atoxigenic *Aspergillus flavus* strains in ability to prevent aflatoxin contamination and production of aflatoxin biosynthetic pathway enzymes. *Appl Environ Microbiol* **60**, 2248–2251.

Cotty, P.J. and Cardwell, K.F. (1999) Divergence of West African and North American communities of *Aspergillus* section Flavi. *Appl Environ Microbiol* **65**, 2264–2266.

Cotty, P.J. and Jaime-Garcia, R. (2007) Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *Int J Food Microbiol* **119**, 109–115.

Cotty, P.J. and Mellon, J.E. (2006) Ecology of aflatoxin-producing fungi and biocontrol of afaltoxin contamination. *Mycotoxin Res* **22**, 110–117.

Cotty, P.J., Bayman, D.S., Egel, D.S. and Elias, K.S. (1994) Agriculture, aflatoxins and Aspergillus. In *The Genus*

*Aspergillus* ed. Powell, K. pp. 1–27. New York: Plenum Press.
Cotty, P.J., Jaime-Garcia, R. and Probst, C. (2008) Etiology and management of aflatoxin contamination. In *Mycotoxins-Detection Methods, Management, Public Health and Agricultural Trade* ed. Leslie, J.F. pp. 287–299. Wallingford, UK: CABI.
Donner, M., Atehnkeng, J., Sikora, R.A., Bandyopadhyay, R. and Cotty, P.J. (2009) Distribution of *Aspergillus* section Flavi in soils of maize fields in three agroecological zones of Nigeria. *Soil Biol Biochem* **41**, 37–44.
Dorner, J.W. (2004a) Biological control of aflatoxin contamination of crops. *Toxin Rev* **23**, 425–450.
Dorner, J.W. (2004b) Biological control of aflatoxin contamination of crops. *J Toxicol Toxin Rev* **23**, 425–450.
Dorner, J.W., Cole, R.J. and Wicklow, D.T. (1999) Aflatoxin reduction in corn through field application of competitive fungi. *J Food Prot* **62**, 650–656.
Egal, S., Hounsa, A., Gong, Y.Y., Turner, P.C., Wild, C.P., Hall, A.J., Hell, K. and Cardwell, K.F. (2005) Dietary exposure to aflatoxin from maize and groundnut in young children from Benin and Togo, West Africa. *Int J Food Microbiol* **104**, 215–224.
Egel, D.S., Cotty, P.J. and Elias, K.S. (1994) Relationships among isolates of *Aspergillus* sect. Flavi that vary in aflatoxin production. *Phytopathology* **84**, 906–912.
Ehrlich, K.C. and Cotty, P.J. (2004) An isolate of *Aspergillus flavus* used to reduce aflatoxin contamination in cottonseed has a defective polyketide synthase gene. *Appl Microbiol Biotechnol* **65**, 473–478.
Ehrlich, K.C., Yu, J. and Cotty, P.J. (2005) Aflatoxin biosynthesis gene clusters and flanking regions. *J Appl Microbiol* **99**, 518–527.
Farombi, E.O. (2006) Aflatoxin contamination of foods in developing countries: implications for hepatocellular carcinoma and chemopreventive strategies. *Afr J Biotechnol* **5**, 1–14.
Garber, R.K. and Cotty, P.J. (1997) Formation of sclerotia and aflatoxins in developing cotton bolls infected by the S strain of *Aspergillus flavus* and potential for biocontrol with an atoxigenic strain. *Phytopathology* **87**, 940–945.
Gong, Y.Y., Hounsa, A., Egal, S., Turner, P.C., Sutcliffe, A.E., Hall, A.J., Cardwell, K. and Wild, C.P. (2004) Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa. *Environ Health Perspect* **112**, 1334–1338.
Hodges, R.J., Dunstan, W.R., Magazini, I.A. and Golob, P. (1983) An outbreak of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in East Africa. *Prot Ecol* **5**, 183–194.
Hodges, R.J., Farrell, G. and Golob, P. (1996) Review of the larger grain borer outbreak in East Africa – rate of spread and pest status. In *Management of Farm Storage Pests in East and Central Africa. Proceedings of East and Central Africa Storage Workshop* ed. Farrell, G., Greathead, A.H., Hill, M.G. and Kibata, G.N. Naivasha, Kenya, 14–19 April 1996. UK: International Institute of Biological Control.
Horn, B. (2003) Ecology and population biology of aflatoxigenic fungi in soil. *J Toxicol Toxin Rev* **22**, 351–379.
Horn, B.W. and Dorner, J.W. (1999) Regional differences in production of aflatoxin B-1 and cyclopiazonic acid by soil isolates of *Aspergillus flavus* along a transect within the United States. *Appl Environ Microbiol* **65**, 1444–1449.
IARC (1982) The evaluation of the carcinogenic risk of chemicals to humans. *IARC Monograph* Supplement 4. Lyon, France: International Agency for Research on Cancer.
IARC (2002) Traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monograph on the Evaluation of Carcinogenic Risk of Chemicals to Humans* Vol. 82. Lyon, France: International Agency for Research on Cancer.
Jaime-Garcia, R. and Cotty, P.J. (2004) *Aspergillus flavus* in soils and corncobs in south Texas: implications for management of aflatoxins in corn-cotton rotations. *Plant Dis* **88**, 1366–1371.
Jaime-Garcia, R. and Cotty, P.J. (2006a) Spatial distribution of *Aspergillus flavus* and its toxigenic strains on commercial cottonseed from South Texas and its relationship to aflatoxin contamination. *Plant Pathol* **55**, 358–366.
Jaime-Garcia, R. and Cotty, P.J. (2006b) Spatial relationships of soil texture and crop rotation to *Aspergillus flavus* community structure in South Texas. *Phytopathology* **96**, 599–607.
Kaaya, A.N. and Kyamuhangire, W. (2006) The effect of storage time and agroecological zone on mould incidence and aflatoxin contamination of maize from traders in Uganda. *Int J Food Microbiol* **110**, 217–223.
Klich, M.A. (2007) *Aspergillus flavus*: the major producer of aflatoxin. *Mol Plant Pathol* **8**, 713–722.
Klich, M.A. and Pitt, J.I. (1988) Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. *Trans Br Mycol Soc* **91**, 99–108.
Krishnamachari, K., Bhat, R., Nagarajan, V. and Tilak, T.B. (1975) Hepatitis due to aflatoxicosis. An outbreak in Western India. *Lancet* **10**, 1061–1063.
Kurtzman, C.P., Horn, B.W. and Hesseltine, C.W. (1987) *Aspergillus nomius*, a new aflatoxin-producing species related to *Aspergillus flavus* and *Aspergillus tamarii*. *Antonie Van Leeuwenhoek* **53**, 147–158.
Lewis, L., Onsongo, M., Njapau, H., Schurz Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L. *et al.* (2005) Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and Central Kenya. *Environ Health Perspect* **113**, 1763–1767.
Lye, M.S., Ghazali, A.A., Mohan, J., Alwin, N. and Nair, R.C. (1995) An outbreak of acute hepatic encephalopathy due to severe aflatoxicosis in Malaysia. *Am J Trop Med Hyg* **53**, 68–72.

McCann, J.C. (2005) *Maize and Grace: Africa's Encounter with a New World Crop, 1500–2000*. Cambridge: Harvard University Press.

Mckean, C., Tang, L., Tang, M., Billam, M., Wang, Z., Theodorakis, C.W., Kendall, R.J. and Wang, J.S. (2006) Comparative acute and combinative toxicity of aflatoxin B1 and fumonisin B1 in animals and human cells. *Food Chem Toxicol* **44**, 868–876.

Muture, B.N. and Ogana, G. (2005) Aflatoxin levels in maize and maize products during the 2004 food poisoning outbreak in Eastern Province of Kenya. *East Afr Med J* **82**, 275–279.

Ngindu, A., Johnson, B.K., Kenya, P.R., Ngira, J.A., Ocheng, D.M., Nandwa, H., Omondi, T.N., Jansen, A.J. *et al.* (1982) Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. *Lancet* **1**, 1346–1348.

Novas, M.V. and Cabral, D. (2002) Association of mycotoxin and sclerotia production with compatibility groups in *Aspergillus flavus* from peanut in Argentina. *Plant Dis* **86**, 215–219.

NTP (1980) First Annual Report on Carcinogens. *Report on Carcinogens*. Research Triangle Park, NC, USA: National Toxicology Program, Department of Health and Human Services.

Pildain, M.B., Vaamonde, G. and Cabral, D. (2004) Analysis of population structure of *Aspergillus flavus* from peanut based on vegetative compatibility, geographic origin, mycotoxin and sclerotia production. *Int J Food Microbiol* **93**, 31–40.

Probst, C. (2005) The S strain of *Aspergillus flavus* is associated with the highly contaminated maize that resulted in deadly aflatoxicoses in Kenya during 2004. *The World Mycotoxin Forum – The Third Conference*. Noordwijk aan Zee, the Netherlands: The International Networking Conference For The Food And Feed Industry.

Probst, C., Njapau, H. and Cotty, P.J. (2007) Outbreak of an acute aflatoxicosis in Kenya in 2004: identification of the causal agent. *Appl Environ Microbiol* **73**, 2762–2764.

Saito, M., Tsuruta, O., Siriacha, P., Kawasugi, S., Manabe, M. and Buangsuwan, D. (1986) Distribution and aflatoxin productivity of the atypical strains of *Aspergillus flavus* isolated from soils in Thailand. *Proc Jpn Assoc Mycotoxicol* **24**, 41–46.

Shephard, G.S. (2003) Aflatoxin and food safety: recent african perspective. *J Toxicol Toxin Rev* **22**, 267–286.

Shephard, G.S. (2008) Risk assessment of aflatoxins in food in Africa. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* **25**, 1246–1256.

Shieh, M.T., Brown, R.L., Whitehead, M.P., Cary, J.W., Cotty, P.J., Cleveland, T.E. and Dean, R.A. (1997) Molecular genetic evidence for the involvement of a specific polygalacturonase, P2c, in the invasion and spread of *Aspergillus flavus* in cotton bolls. *Appl Environ Microbiol* **63**, 3548–3552.

Tandon, B.N., Krishnamurthy, L., Koshy, A., Tandon, H.D., Ramalingaswami, V., Bhandari, J.R., Mathur, M.M. and Mathur, P.D. (1977) Study of an epidemic of jaundice, presumably due to toxic hepatitis, in Northwest India. *Gastroenterology* **72**, 488–494.

Turner, P.C., Sylla, A., Gong, Y.Y., Diallo, M.S., Sutcliffe, A.E., Hall, A.J. and Wild, C.P. (2005) Reduction in exposure to carcinogenic aflatoxins by postharvest intervention measures in West Africa: a community-based intervention study. *Lancet* **365**, 1950–1956.

Vaamonde, G., Patriarca, A., Pinto, V.F., Comerio, R. and Degrossi, C. (2003) Variability of aflatoxin and cyclopiazonic acid production by *Aspergillus* section Flavi from different substrates in Argentina. *Int J Food Microbiol* **88**, 79–84.

Van Egmond, H.P. and Jonker, M.A. (2004) Worldwide regulations on aflatoxins – the situation in 2002. *Toxin Rev* **23**, 273–293.

Wang, J.-S. and Tang, L. (2004) Epidemiology of aflatoxin exposure and human liver cancer. *Toxin Rev* **23**, 249–271.

Wicklow, D.T. and Cole, R.J. (1982) Tremorgenic indole metabolites and aflatoxins in sclerotia of *Aspergillus Flavus* – an evolutionary perspective. *Can J Bot* **60**, 525–528.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M. and Aggarwal, D. (2004) Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *Am J Clin Nutr* **80**, 1106–1122. sample sites in 2006, nonaflatoxicosis provinces.


 